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To: [Dana Davoli/R10/USEPA/US@EPA](#)
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Subject: Re: Fw: Portland Harbor HH bass composites
Date: 10/02/2007 10:42 AM

Dana,
The guidance specifically recommends using equal amount of homogenate from individual fish to create the composite (see Figure 7-1. Preparation of fish fillet composite homogenate sample and p. 7-15: "Composite homogenates should be prepared from equal weights of individual homogenates."). If the recommended approach is not followed, you will have unequal sample size in your composites, which will not provide an unbiased estimate of the mean. If the guidance for creating composite samples is followed, I would have no problem using the 0.75 length factor for creating acceptable composites. Note that the guidance also recommends archiving homogenate from each individual fish.
Jay

Davoli.Dana@epamail.epa.gov wrote:

```
> Jay, I do not think the guidance recommends using unequal amounts from
> each fish to create the composite. I have included the language from the
> guidance below so you can make your own interpretation. The site is:
> http://www.epa.gov/waterscience/fishadvice/volumel/vlch6.pdf
>
>
> 6.1.1.6 Sample Type
> (Page 6-18)
> Note: Composite samples are homogeneous mixtures of samples from two or
> more individual organisms of the same species collected at a particular
> site and
> analyzed as a single sample. Because the costs of performing individual
> chemical
> analyses are usually higher than the costs of sample collection and
> preparation,
> composite samples are most cost-effective for estimating average tissue
> concentrations of target analytes in target species populations. Besides
> being
> cost-effective, composite samples also ensure adequate sample mass to
> allow
> analyses for all recommended target analytes. A disadvantage of using
> composite samples, however, is that extreme contaminant concentration
> values
> for individual organisms are lost.
>
> In screening studies, EPA recommends that states analyze one composite
> sample for each of two target species at each screening site. Organisms
> used in
> a composite sample:
>   Must all be of the same species
>   Should satisfy any legal requirements of harvestable size or weight,
>   or at least
> be of consumable size if no legal harvest requirements are in effect.
>   Should be of similar size so that the smallest individual in a
>   composite is no
>   less than 75 percent of the total length (size) of the largest
>   individual. Should be collected at the same time (i.e., collected as
>   close to the same time as possible but no more than 1 week apart) [Note:
>   This assumes that a
>   sampling crew was unable to collect all fish needed to prepare the
>   composite
>   sample on the same day. If organisms used in the same composite are
>   collected on different days (no more than 1 week apart), they should be
>   processed within 24 hours as described in Section 7.2 except that
>   individual
>   fish may have to be filleted and frozen until all the fish to be
>   included in the
>   composite are delivered to the laboratory. At that time, the composite
>   homogenate sample may be prepared.]
>   Should be collected in sufficient numbers to provide a 200-g
>   composite
> homogenate sample of edible tissue for analysis of recommended target
> analytes.
>
> Individual organisms used in composite samples must be of the same
> species
> because of the significant species-specific bioaccumulation potential.
> Accurate
> taxonomic identification is essential in preventing the mixing of
> closely related
> species with the target species. Note: Individuals from different
> species should
> not be used in a single composite sample (U.S. EPA, 1989d, 1990d).
>
> For cost-effectiveness, EPA recommends that states collect only one size
> class
> for each target species and focus on the larger individuals commonly
```

> harvested
 > by the local population. Ideally, each composite sample for a specific
 > species
 > should contain the same number of individual fish and the individuals
 > within each
 > target species composite should be of similar size within a target size
 > range so
 > that the composite samples for a particular species are comparable over
 > a wide
 > geographic area. This is particularly important when states want to
 > compare data
 > on an individual species that might be used to establish a statewide
 > advisory.
 >
 > For persistent chlorinated organic compounds (e.g., DDT, dioxin, PCBs,
 > and
 > toxaphene) and methylmercury, the larger (older) individuals within a
 > population
 > are generally the most contaminated (Phillips, 1980; Voiland et al.,
 > 1991). As
 > noted earlier, this correlation between increasing size and increasing
 > contaminant
 > concentration is most striking in freshwater finfish species but is less
 > evident in
 > estuarine and marine species. Size is used as a surrogate for age, which
 > provides some estimate of the total time the individual organism has
 > been at risk
 > of exposure. Therefore, the primary target size range ideally should
 > include the
 > larger individuals harvested at each sampling site. In this way, the
 > states will
 > maximize their chances of detecting high levels of chemical
 > contamination in the
 > single composite sample collected for each target species. If this ideal
 > condition
 > cannot be met, the field sampling team should retain individuals of
 > similar length
 > that fall within a secondary target size range.
 >
 > Individual organisms used in composite samples should be of similar size
 > (WDNR,
 > 1988). Note: Ideally, for fish or shellfish, the total length (or size)
 > of the smallest
 > individual in any composite sample should be no less than 75 percent of
 > the total
 > length (or size) of the largest individual in the composite sample (U.S.
 > EPA,
 > 1990d). For example, if the largest fish is 200 mm, then the smallest
 > individual
 > included in the composite sample should be at least 150 mm. In the
 > California
 > Mussel Watch Program, a predetermined size range (55 to 65 mm) for the
 > target
 > bivalves (*Mytilus californianus* and *M. edulis*) is used as a sample
 > selection
 > criterion at all sampling sites to reduce size-related variability
 > (Phillips, 1988).
 > Similarly, the Texas Water Commission (1990) specifies the target size
 > range for
 > each of the recommended target fish species collected in the state's
 > fish
 > contaminant monitoring program.
 >
 > Individual organisms used in a composite sample ideally should be
 > collected at
 > the same time so that temporal changes in contaminant concentrations
 > associated with the reproduction cycle of the target species are
 > minimized.
 >
 > Each composite sample should contain 200 g of tissue so that sufficient
 > material
 > will be available for the analysis of all recommended target analytes. A
 > larger
 > composite sample mass may be required when the number of target analytes
 > is
 > increased to address regional or site-specific concerns. However, the
 > tissue
 > mass may be reduced in the Tier 2 intensive studies (Phase I and II)
 > when a
 > limited number of specific analytes of concern have been identified (see
 > Section
 > 7.2.2.9). Given the variability in size among target species, only
 > approximate
 > ranges can be suggested for the number of individual organisms to
 > collect to
 > achieve adequate mass in screening studies (U.S. EPA, 1989d; Versar,
 > 1982).
 > For fish, 3 to 10 individuals should be collected for a composite sample
 > for each
 > target species; for shellfish, 3 to 50 individuals should be collected
 > for a composite
 > sample. In some cases, however, more than 50 small shellfish (e.g.,
 > mussels,
 > shrimp, crayfish) may be needed to obtain the recommended 200-g sample
 > mass.
 > Note: The same number of individuals should be used in each composite
 > sample
 > for a given target species at each sampling site.
 >
 > Deviations from the recommended study design have implications that may
 > make

> the statistical analyses more complicated. The statistical methods for
> analyzing
> composite samples are made tractable and easier-to-use by simplifying
> the study
> design. Using equal numbers of fish in replicate composite samples is
> one way
> to do this. For example, with equal numbers of fish, the arithmetic
> average of the
> replicate composite measurements is an unbiased estimator of the
> population
> mean. When unequal numbers are used, the arithmetic average is no longer
> unbiased. Instead, a weighted average of the composite measurements is
> calculated, where the weight for each composite reflects the number of
> fish it is
> made up of. Oftentimes fish are lost or damaged prior to compositing.
> When
> several fish are damaged or lost, the allocation of the remaining fish
> to
> composites may be reconfigured to allow equal numbers of fish in
> composites. If
> this is not possible, care should be taken to adjust the statistical
> procedures to
> account for the unequal allocations.

>
> The use of sizes of fish exceeding the size range recommended for
> compositing
> may introduce more variability. If it is the size range within each
> composite that
> is broadened (e.g., 100-200 mm instead of 150-200 mm), the variability
> within the
> composite may increase. If additional composites are made with fish
> exceeding
> the recommended size ranges (e.g., adding composites of fish of size
> 300-450
> mm when the target size is no more than 250 mm), this may increase the
> variability between composites of different size ranges. Overall
> inferences made
> from composites of different size ranges will have increased variability
> associated
> with them (e.g., wider confidence intervals).

>
> Differences in the numbers of replicates at different sampling locations
> may
> complicate any comparisons to be made between locations or overall
> conclusions
> to be obtained by combining the results from different sampling
> locations. As with
> unequal numbers of fish in composites, unequal numbers of replicate
> samples
> complicate the statistical calculations. The appropriate weighted
> estimates should
> be used when combining information from different sampling locations.
> Consider,
> for instance, a state that monitors five lakes each year. If the state
> uses the same
> target fish species, the same number of fish per composite and the same
> size
> ranges, the overall mean level of contamination will be a
> straightforward average
> over the five locations if the same number of replicates are used at
> each location.
> However, if unequal numbers of replicates are used, the information
> contributed
> by each location is not the same and must be weighted accordingly.

>
> As alluded to above, one limitation of using composite samples is that
> information
> on extreme levels of chemical contamination in individual organisms is
> lost.
> Therefore, EPA recommends that the residual individual homogenates be
> saved
> to allow for analyses of individual specimens if resources permit
> (Versar, 1982).
> Analysis of individual homogenates allows states to estimate the
> underlying
> population variance which, as described in Section 6.1.2.6, facilitates
> sample size
> determination for the intensive studies. Furthermore, individual
> homogenates
> may also be used to provide materials for split and spike samples for
> routine QC
> procedures either for composites or individual organisms (see Section
> 8.3). The
> circumstances in which the analysis of individual fish samples might be
> preferred
> over the analysis of composite samples is described in more detail in
> Appendix C.
> Recommended sample preparation procedures are discussed in Section 7.2

Jay Field
<Jay.Field@noaa.
gov>

10/02/2007 09:43
AM

Dana Davoli/R10/USEPA/US@EPA To
cc

Subject
Re: Fw: Portland Harbor HH bass
composites

```
> Dana,  
> does the guidance recommend using unequal amounts from each fish to  
> create the composite?  
> Jay  
  
> Davoli.Dana@epamail.epa.gov wrote:  
> Jay, for the PH RI Round 1 and this round of sampling we have been  
> following the guidance given in USEPA "Guidance for Assessing  
> Chemical  
> Contaminant Data for Use in Fish Advisories". This guidance  
> recommends  
> using the 0.75 length criteria for composites. We are using the  
> entire  
> fish, not an aliquot. Sex of the fish has not been considered.  
> Thanks!  
  
Jay Field  
<Jay.Field@noaa.gov>  
  
To Dana Davoli/R10/USEPA/US@EPA  
  
10/02/2007 09:20 AM  
cc Chip  
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```

Subject
bass
Re: Fw: Portland Harbor HH
composites

Dana,
If you are compositing without taking equal aliquots from individual fish to create the composite sample, then you should consider using weight rather than length to estimate the relative contribution of small

and large fish. For example, for the first composite on your spreadsheet, the proportion of smallest/largest is 0.75 for length and less than 0.4 for weight.
Jay

PS Did they determine the sex of individual fish and consider that information in creating the composite samples?

Davoli.Dana@epamail.epa.gov wrote:

Mike Poulsen and I discussed bass compositing with Laura Kennedy from Kennedy-Jenks yesterday. We have compiled bass composites that would meet the objectives for the human health risk assessment. We would

like

input as to whether these composites will also meet the other

objectives

for bass in the RI/FS, including the ecological risk assessment, the food web model, and identification of sources of contamination. Please let us know if these proposed composites are OK by COB Thursday,

October

4. Thanks!

----- Forwarded by Dana Davoli/R10/USEPA/US on 10/02/2007 08:43 AM

"POULSEN Mike"

<POULSEN.Mike@de

q.state.or.us>

To

Davoli/R10/USEPA/US@EPA Dana

10/01/2007 04:19

cc

PM

Subject

bass Portland Harbor HH

composites

>
> largest fish of 0.75 or greater. This was to avoid a
> situation where
>
> one
>
> large fish would dominate the concentration in a composite,
> and to
> minimize size as a variable that needs to be considered in
> evaluating
> the data. Fish that are longer generally weigh more, and are
> generally
> older than smaller fish. Older fish are more likely to have
>
> accumulated
>
> chemicals of interest. Larger fish are more desirable as
> food fish.
>
> For
>
> these reasons, including larger fish in the composite meets
> the needs
>
> of
>
> the human health risk assessment. However, we understand
> that larger
> fish may not be appropriate for the ecological risk
> assessment.
>
> The selection criteria were not strictly applied in Round 1.
> Many of
>
> the
>
> fish included in composites were greater than 355 mm. The
> criterion of
> 0.75 was not always met. EPA and LWG accepted the composite
> approach
>
> in
>
> Round 1, acknowledging that not all the criteria were met.
> We
>
> therefore
>
> do not feel strictly bound by the criteria in Round 3.
>
> Using the proposed compositing approach, four of the reaches
> do not
>
> meet
>
> the 0.75 criterion: RM 6 East (0.74), RM 6 West (0.71), RM 8
> West
> (0.70), and RM 10 West (0.64). If the maximum length of 403
> mm is
> removed from RM 10 West and replaced with the 251 mm value,
> the
>
> revised
>
> ratio is 0.77.
>
> In Round 1, the mean length in a composite was generally
> less than 300
> mm. In Round 3, the mean length is generally greater than
> 300 mm,
> particularly in upstream sampling areas. The two areas with
> the
>
> largest
>
> difference between sides of the river are RM 6 (271 mm East
> v. 315 mm
> West) and RM 11 (271 mm East v. 338 mm West). It is not
> clear if
> differences of this size in fish would confound comparisons
> of areas.
> For fish of similar sizes, differences in concentrations may
> be
>
> related
>
> to proximity to source areas. However, if one of the reasons
> for the
> differences in concentrations is the size (age) of fish,
> this could
> confound a determination of sources.
>
> - Mike
> <<HH bass composites R3B.xls>>
> (See attached file: HH bass composites R3B.xls)
>
>
>
> --
> Jay Field
> Assessment and Restoration Division
> Office of Response and Restoration, NOAA

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